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International Biodeterioration & Biodegradation 61 (2008) 194–198

INTERNATIONAL BIODETERIORATION & BIODEGRADATION

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Short communication

Ultrastructural characteristics of cell wall disintegration of *Pinus* spp. in the windows of an old Buddhist temple exposed to natural weathering ☆

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Received 20 September 2007; received in revised form 5 November 2007; accepted 5 November 2007

Abstract

An ultrastructural study was conducted to examine the disintegration pattern of cell wall layers during natural weathering. Pine wood samples from the wooden windows in an old Buddhist temple in the Republic of Korea were investigated by transmission electron microscopy (TEM) and ultraviolet (UV) microscopy. The first disintegration during the weathering process occurred in the cell corner middle lamella and extended to the compound middle lamella. Dissolution of warts and loosening of the S3 layer occurred almost simultaneously with the degradation of the middle lamella. After degradation and separation of the S3 layer from the S2 layer, defibrillation appeared in the S1 layer. The S2 layer was the most resistant against weathering. However, the S2 layer was also defibrillated at the later stage of weathering. Defibrillation in the S2 layer at the last stage of weathering suggested that lignin was degraded first, followed by hemicellulose and cellulose. UV microscopic work showed that lignin in tracheids was linearly decreased from the surface to the inner parts of the cells. TEM and UV microscopy exhibited that lignin in the middle lamella was much more susceptible to weathering than that in the secondary wall, thus suggesting the chemistry of lignin in the middle lamella to be different from that in the secondary walls.

Keywords: Cell wall disintegration; Lignin removal; Natural weathering; TEM; UV microscopy

1. Introduction

Weathering can be defined as the degradation of wood exposed above ground (Williams et al., 2001). Typical modifications caused by weathering include discoloration, erosion, and loss of mass. Degradation of wood by weathering occurs mainly on the surface (Pandey and Pitman, 2002). Other climatic factors such as rain, heat, and pollutants are also involved in the degradation of exposed wood (Plackett et al., 1992). However, biotic agents such as wood decay fungi and molds are not considered to be the primary agents of weathering (Williams et al., 2001).

Although much is known about the weathering of wood, information on the disintegration of the cell wall by weathering at the ultrastructural levels is limited. Furthermore, few topochemical studies have been conducted to assess the removal of lignin from the cell walls during weathering. Most of the anatomical studies on the degradation of weathered wood surfaces appear to have been conducted by either light or scanning electron microscopy (Borgin, 1971; Owen et al., 1993; Evans et al., 1996), and only a few studies have utilized transmission electron microscopy (TEM) (Kuo and Hu, 1991; Singh and Dawson, 2003).

The primary reactions of photolytic degradation of wood components are well described (Fengel and Wegener,

☆Information on the disintegration of wood cell wall during natural weathering at the ultrastructural levels is limited. In particular, information on the disintegration of archaeological woods by long-term weathering process is very limited. The present work showed clearly the disintegration pattern of the cell wall layers during natural weathering. Our work provides better understanding of lignin removal from cell wall layers during natural weathering process by TEM and UV microscopy.

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doi:10.1016/j.ibiod.2007.11.002

1984). Lignin is considered to be an excellent absorber of ultraviolet (UV) irradiation and energy in the range of 200–400 nm initiates degradation processes of this polymer. However, UV rays also attack all wood polysaccharide components, including cellulose and hemicellulose (Tylli et al., 1993; Evans et al., 1996). The loss of weight, reduction of the α-cellulose content, and degree of polymerization reflect the degradation of cellulose under the influence of UV rays.

The present work was undertaken to better understand the disintegration of cell wall layers in a wooden cultural property during long-term exposure of dry archeological woods to outdoors conditions. In particular, the topochemistry of lignin removal from the cell wall layers was examined using TEM and UV microscopy.

2. Materials and methods

Materials were obtained from wooden windows of an old Buddhist temple located in Suncheon, Republic of Korea. This temple was designated as a cultural property by the Ministry of Culture and Tourism of the Government of the Republic of Korea. Wooden windows facing south were thought to have been exposed to natural weather conditions for over 100 years. Wood specimens were identified as Japanese red pine (*Pinus densiflora*). Small pieces of wood from five different locations on the windows showing signs of surface deterioration were carefully removed. After fixation with 3% glutaraldehyde in sodium cacodylate buffer, wood samples were embedded in Spurr's epoxy resin using standard procedures. Thin sections prepared by rotary microtome were stained with toluidine blue and observed under a light microscope (LM). Some sections were also observed under a polarized microscope without staining. Transverse, ultra-thin sections (100 nm thickness) were prepared using an ultramicrotome and a diamond knife and stained with potassium permanganate to examine lignin distribution (Donaldson, 1992). Samples were examined with a JEOL 1010 TEM.

For UV microscopic observation, semi-thin transverse sections (1 μm thick) from Spurr resin embedded blocks were prepared using an ultramicrotome with a diamond knife. The sections were transferred to quartz slides, immersed in a drop of non-UV absorbing glycerine, and covered with quartz cover slips. UV-absorbance spectra were recorded using a Zeiss UMSP 80 micro-spectrophotometer. The determination of image profiles was conducted at a constant wavelength of 280 nm using the APAMOS scan program (Zeiss). The scan program digitized rectangular tissue portions with a local geometrical resolution of 0.25 μm^2 and a photometrical resolution of 4096 gray scale levels, converted into 14 basic colors representing the measured absorbance intensities (Koch and Kleist, 2001).

3. Results and discussion

The information presented here has to be viewed with the following consideration in mind. The observations presented are based on the examination of small wood pieces taken from five different locations on the windows and the micrographs illustrated are representative of all samples examined. The Buddhist temple is a wooden cultural property, which posed restrictions on the number and size of wood pieces that could be sampled.

As expected, the most severe degradation of the tracheids was localized to the surface region. In contrast, the inner part appeared to have remained intact. The degradation of tracheids was found on the surface of about 20 cells on average. Due to low penetration of UV rays (Fengel and Wegener, 1984), photodegradation in wood is more or less localized in a surface reaction. LM analysis showed that the main anatomical change in the tracheids was the degradation of the middle lamella (Fig. 1), resulting in the separation of tracheids. However, the secondary wall of the tracheids in the surface region exhibited birefringence under polarized light (Fig. 2). Fig. 3 shows a section of modern Japanese red pine with intact tissues.

TEM analysis of undegraded region showed that the inner portion of the tracheids did not show any obvious changes with respect to their electron densities. Cell corners displayed the highest contrast in comparison with the S1, S2, and S3 layers, thus reflecting the different lignin content in the various wall layers. In addition, numerous warts covered the S3 layer (Fig. 4).

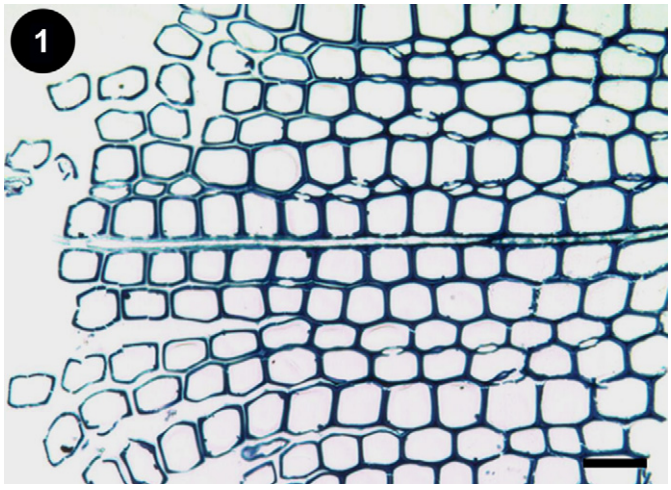

Fig. 1. General aspects of cell wall disintegration during natural weathering. Note that the degradation was limited to the surface. LM, Toluidine blue staining. Bar = 50 μm.

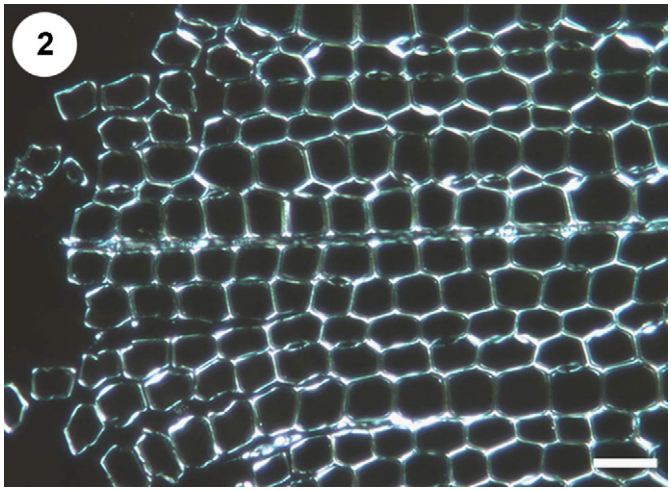

Fig. 2. Birefringence in the secondary wall of tracheids. Polarized microscopy. Bar = 50 μm.